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THE EARLY DEVELOPMENT OF LEPIDOSTEUS OSSEUS

BY

ALBERT CHAUNCEY EYCLESHYMER

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THE EARLY DEVELOPMENT OF LEPIDOSTEUS OSSEUS

BY

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ASSISTANT PROFESSOR OF ANATOMY

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THE EARLY DEVELOPMENT OF LEPIDOSTEUS OSSEUS

ALBERT CHAUNCEY EYCLESHYMER

INTRODUCTORY

CERTAIN phases of the early development of Lepidosteus have been described and depicted by Balfour and Parker, Beard, Dean, and others. These investigators studied preserved material only, and arrived at widely different conclusions regarding certain fundamental features of development. Balfour and Parker (1882) and Beard (1889) consider the egg as holoblastic, while Dean (1895) maintains that it is meroblastic. Thus it is evident that either the egg of Lepidosteus is quite unlike that of any other known vertebrate, or that the character of its cleavage has been misinterpreted.

In 1897 the writer obtained an abundance of the embryological material and found that no one had described the cleavage as presented by the living egg; attention has been called to this fact in a preliminary note (1899). The detailed study of the preserved material not only has confirmed the deductions made, but also has revealed other new features of development, which will be described in the following pages.

The greater part of the material for this study was obtained June 13, 1897, from La Belle Lake, near Oconomowoc, Wis. Some material was obtained June 20, 1900, from Sand Lake, near Nottawa, Mich. The fish were taken by spearing and, although more or less mangled, the eggs and sperm were in most cases in such an excellent condition that artificial fertilization was easily produced. The eggs and sperm obtained by pressure ("stripping") were more favorable for fertilization than when got by excision. The eggs were fertilized in earthen dishes, in which they remained until the embryos hatched.

From this material several series were preserved, comprising the stages between the unsegmented egg and the 25 mm. larva. In preserving the eggs the following fixing fluids were used: formaline in 8 to 10 per cent. aqueous solution, which, followed by hæm-alum surface-staining, clearly defined the cleavage grooves; chrom-acetic, to which a little 1 per cent. osmic acid was added, gave most excellent results; corrosive sublimate, saturated aqueous solution, plus 1 per cent. glacial acetic acid, proved to be most satisfactory for sectioning and for section-staining; picro-acetic and picro-sulphuric, while fairly good for the larval stages, caused swelling and distortion of the eggs often entirely obscuring the cleavage grooves.

Since the writer's observations on the general and breeding habits of Lepidosteus add nothing of importance to the records of other observers, the study may be introduced by a general description of the surface changes going on between the freshly fertilized egg and the larva at the time of hatching.

OUTLINE OF DEVELOPMENTAL PHASES UP TO TIME OF HATCHING AT 20° C.

In nearly all the eggs the first cleavage begins about one and one-half to two hours after fertilization. At the end of six hours a cap containing 250–300 cells has been formed. Four hours later the blastodermic cap extends downward over a distance equal to one-third the long axis of the egg. About the time the rim of the extending blastodisc reaches the region of the equator, a faint linear tract grows out from the margin of the blastodisc toward the upper pole. This tract, which is lighter than the surrounding parts, represents the *Anlage* of the forthcoming embryo. At the end of twenty-four hours the blastopore is reduced to about one-third the diameter of the egg, and four hours later is barely visible; the embryo meantime becomes well defined. In from forty-five to forty-eight hours after fertilization faint lateral swellings mark the beginnings of the optic vesicles, neither the cephalic nor the caudal end of the embryo is as yet free from the yolk, nor is there any trace of the pronephric ducts. In about fifty-six hours the embryo has reached a length of about 2½ mm.; the cerebral vesicles are plainly marked, the mid-brain being especially distinct; the bases of the adhesive disks are discernible; the tail of the embryo is becoming free from the yolk, while the pronephric ducts and mid-dorsal myotomes are visible. About eighty hours after fertilization the first movements of the larva are observed; these movements are but momentary, and are confined to the anterior mid-dorsal myotomes. Sections of the embryos of this period show that the appearance of these movements is practically coincident with the differentiation of fibrillæ in the myoblasts. The envelopes at this time measure 4–5 mm. in diameter, leaving a considerable space around the larva. During the fourth day the larva attains a length of 6–7 mm. When freed from the envelopes it strives to maintain an upright position; if it be accidentally turned on its side, it makes a few brisk movements and attains the position sought. It is of interest to note that the larval Amia of a corresponding stage is wholly unable to maintain or regain an upright position. On the fifth day the larva has reached a length of 8–9 mm. and is more active. If the envelopes be removed, it makes vigorous efforts to swim about, but is barely able to sustain its heavy load of food-yolk. The adhesive disks are now functional, and the larva clings to various objects, often so tenaciously that they are not detached by the action of the killing fluids.

Sometime during the sixth day the larva breaks through the surrounding envelopes, which have now become soft and flimsy, and swims vigorously about, rarely coming to rest until some object is found to which it can easily adhere. It is noticeable that the larva is much stronger and more active than the larva of Amia at a corresponding stage, being able to support and easily move its mass of food-yolk. At this time the yolk-sac has elongated and become slightly narrowed at its middle. The beginnings of the operculæ can be distinguished. Pigment is present on the upper surface of the yolk; it is likewise scattered over the head, along the sides of the body, and faint traces are seen in the retinæ.

The observations made by different investigators indicate that the time of hatching varies from three to nine days, as shown in the following table:

Authority	No. Days, Fertilization to Hatching
Garman and Agassiz	8
Mark	7
Beard	9
Beard	7
Dean	8
Perry (per Dean)	5
Fülleborn	3
Eycleshymer	6

It is a significant fact that the longest periods indicated were those in which the eggs were laid during the month of May, while in the other cases cited the eggs were laid during the month of June. It is therefore probable that the explanation of this variation is to be found, in part, at least, in the differences in water temperature.

A description of the changes which the larva undergoes after hatching would be of little value in the present paper, but for the convenience of those who may obtain isolated stages a brief tabulation of some of the more conspicuous changes is here given:

Age in Hours	Length in mm.	Stage of Development
0	.....	Fertilization, beginning of
1.40	.....	First cleavage, beginning of
2.15	.....	Second cleavage, beginning of
2.48	.....	Third cleavage, beginning of
3.25	.....	Fourth cleavage, beginning of
4.00	.....	Fifth cleavage, beginning of
4.36	.....	Sixth cleavage, beginning of
5.10	.....	Late cleavage, 100 to 200 cells
5.45	.....	Blastula, 200 to 300 cells
10.00	.....	Gastrulation, beginning of
18.00	.....	Blastodisc extends over one-third diameter of egg
24.00	.....	Embryo appears, blastopore one-third diameter of egg
28.00	.....	Blastopore closes
45.00	.....	Optic vesicles distinguishable
80.00	4–5	Embryo begins to move
145.00	9–10	Embryo hatches
185.00	10–11	Pectoral fins appear
312.00	13–14	Pelvic fins appear
480.00	21–22	Yolk absorbed

CLEAVAGE

THE term "cleavage," as here used, covers the period intervening between the formation of the first cleavage groove and the beginning of gastrulation. The description which follows is based upon a detailed examination of the surface phenomena as presented in both living and preserved material, together with a study of serial sections of corresponding stages.

Immediately after fertilization the villi of the external envelope of the egg become attached at their peripheral ends to the first object touched; the egg is thus supported in a fixed position until the larva escapes. The egg is oval in profile like that of Amia, although a trifle larger, measuring in its longest diameter, including membranes, 3–3.5 mm.; in its shortest, 2.5–2.75 mm. The upper portion of the egg, for which the term "calotte" seems preferable, is a pale creamy gray, which shades off, in a zone midway between the upper pole and the equator, into the pale drab of the yolk, as indicated in Fig. 1 (Plate XVII). Although the calotte usually appears circular in outline when viewed from the upper pole, as in Figs. 11–20 (Plate XVII), it is sometimes oval and frequently irregular. No features of contour or pigmentation have been detected which would enable one to predict the direction of any of the early cleavage grooves, or the direction of the future embryonic axes. Near the center of the calotte a single micropylar orifice is present, as described by Mark, Dean, and others. Its position with reference to the cleavage grooves is variable.

A vertical section of a mature ovarian egg is represented in Fig. 30, which shows the extent and relation of the finely granular germinal part to the coarsely granular nutritive portion. It is to be especially emphasized that this condition is a most peculiar one and, so far as the writer is aware, does not occur elsewhere among the Icthyopsida. In this egg there is added to the blastodisc or calotte of the Teleostean egg a conical prolongation which extends to, or beyond, the equator of the egg, giving to the finely granular portion a pear-shaped outline. Around the margin of this conical prolongation are scattered numerous small vesicular structures, as shown in Figs. 30, 31, 32, 34.

I have carefully and repeatedly read the descriptions given by the various writers, but have been unable, save in a single instance, to find any reference to this strikingly peculiar condition. In Plate V, Fig. 1, of Mark's (1890) illustrations a section of the ovarian egg is represented which suggests that he observed something of the same sort. Concerning its significance nothing is said beyond the brief reference given in the explanation of the figures, which reads as follows: "The finely granular and vacuolated portion of the yolk beneath the germinative vesicle is in the center of the egg."

First cleavage.— The first cleavage, as in Amia, is foreshadowed by a flattening of the upper pole of the egg, which in reality is a thinning of the calotte at its center. This soon changes to a wide shallow furrow, which in turn gives rise to a narrow deep fissure or cleavage groove. The groove soon extends in opposite directions over the surface of the egg, giving rise to the two blastomeres as shown in Figs. 2, 11. Its progress through the calotte is rapid and continuous, requiring but five to eight minutes to traverse the distance from center to periphery; beyond the margin of the calotte the groove travels at a gradually decreasing rate, as shown in Figs. 2–5 and 11–15, until it reaches a zone lying somewhat below the equator, where it either fades out or terminates abruptly. In most cases it seems to reach its maximum extension at the time of the sixth or seventh cleavage. Its extent seems to be fairly constant, and in no case under my observation have the ends of this groove extended to the lower pole or even to its vicinity. Neither have I ever found them terminating in or near the margin of the calotte. Variations in the formation of this furrow are seldom observed. Among fifty preserved eggs but seven were found in which the first two blastomeres showed marked difference in size. In no case has the writer observed the pronounced inequality which so frequently occurs in the cleavage of the egg of the Teleost (Coregonus, Serranus, Amiurus).

A vertical section of an egg just before the appearance of the second cleavage is shown in Fig. 32 (Plate XVIII). The line of the section is indicated by the dotted meridional line in Fig. 21. The groove has cut deeply into the germinal area at its center, but as it passes toward the margin it decreases in depth, as shown in Fig. 33 (Plate XVIII), which is taken along the lower dotted line of Fig. 21. The blastodisc has undergone no visible changes in form or extent. The plane of the dividing nuclei is indicated in the figure; it will be observed that these nuclei lie well up toward the surface of the blastodisc.

Dean has described a section of an egg in a stage slightly earlier. Of this he writes as follows: "Sections, however, indicate that at this stage germ-disc and yolk are more intimately connected, and in Plate II, Fig. 21, it will be seen that the protoplasm of the animal pole is hardly to be separated from the coarsely granular yolk until nearly in the equatorial region of the egg. In the figure the nucleus is seen dividing at about one-third the diameter of the egg from the surface." The first reading of the above led me to believe that, if the cleavage grooves originated at the point shown in Dean's Fig. 21, Plate II, they must either become completely obliterated centrally, or extend much deeper than Dean had described for the succeeding stages. Moreover, it was at first inferred that Dean had observed and described the peculiar conical elongation of the blastodisc. A careful examination of the drawing of the section Fig. 21, Plate II, upon which Dean based the above statement, plainly reveals the fact that, instead of its being a vertical section, as stated by the author, it is cut in an oblique plane, thereby giving the appearance described. In the next figure (Fig. 22) given by Dean the position of the nuclei is more accurately represented, and it is, moreover, apparent that the peculiar form of the blastodisc has been overlooked.

Second cleavage.—This cleavage begins about thirty minutes after the appearance of the first. It is always foretold by a rapid closing of the first cleavage groove. The two grooves of this cleavage usually begin at the same point and extend in opposite directions, thus giving rise to an apparently continuous groove. In the egg followed in detail (Fig. 3) the groove on one side arose slightly in advance of that on the opposite side, as indicated by the times affixed. The furrows progress at the same rate as the first, passing from the center to the margin of the blastodisc in a few minutes; beyond the margin their progress is greatly retarded, and not until several hours later do the grooves reach their maximum extension, finally terminating in a zone near the equator. Variations in the position of the grooves sometimes occur; instead of forming right angles with the first they form acute or obtuse angles, thereby giving rise to decidedly unequal blastomeres. Again, the grooves, instead of passing in meridional planes, may converge toward the first furrow, but rarely is this convergence so pronounced that the two furrows fuse. The variations are more numerous than in Amia, but far less frequent than in the Teleosts (Amiurus, Serranus, Coregonus). A shifting of the blastomeres is frequently observed, although not to such an extent as in the Amphibia (Necturus, Amblystoma, Diemyctylus, Rana, Bufo, Acris).

Sections show the depth of these furrows to be about the same as that of the first. It is a noticeable fact that the cleavage grooves, instead of following a given plane, become more or less tortuous, as shown in Fig. 34 (Plate XVIII). An intercellular space is frequently present at the line of intersection of the first and second cleavage grooves. From the position of the elongated nuclei of the four-cell stage one can predict the planes of the ensuing divisions. In all cases in which the horizontal sections passed through the nuclei, the latter were as indicated in Fig. 25 (Plate XVIII).

Balfour and Parker figure these grooves as extending to the immediate vicinity of the lower pole. The description by Beard leads to the conclusion that they actually reach the lower pole. Dean states that this cleavage "is expressed in the germ-disc only, and like the former furrow could not be traced into the yolk region of the egg." Among all the eggs which I have examined I have never found one in which these furrows extended as far as described by Balfour and Parker, neither have I observed the condition described by Dean.

Third cleavage.—The four grooves which constitute this cleavage are, in all cases examined, vertical; they usually occupy the positions shown in Figs. 4, 19, 20 (Plate XVII), conforming in general to the pattern of the Teleost. Sometimes, however, one observes the condition shown in Fig. 13 (Plate XVII), where the grooves approach a meridional position. The order of their appearance is irregular, beginning in this or that quadrant and without sequence, only a few minutes elapsing between the origin of the first and the last. In the egg followed in detail (Fig. 4), the times at which these furrows form are indicated by the numerals affixed. They travel at the same rate as those of the second cleavage, and finally terminate at about the same latitude.

Three horizontal sections of a blastodisc at this stage are represented in Figs. 26, 27, 28 (Plate XVIII). The sections are taken at different levels, and show the extent of the cleavage grooves long before they are apparent on the surface. It will be noticed that the intercellular space has enlarged. In other eggs much larger spaces are sometimes found. While as yet there is nothing which could be called a cleavage cavity, it nevertheless seems probable that this space may be its forerunner, or at least that it later contributes to its formation. It is worthy of remark that in Amia the cleavage cavity takes its origin in an entirely different manner, being formed by the union of a number of spaces which are present in the egg as early as the first cleavage. Fig. 34 (Plate XVIII) represents a vertical section along the plane of the dotted line in Fig. 23; it shows the relative depth of the furrows at this time. The first groove has progressed toward the center of the egg, but a comparison with Fig. 32 shows that its rate after reaching the depth shown in Fig. 32 is exceedingly slow.

Concerning the extent of the grooves of this cleavage, authors are again at variance. Beard believes that they extend to the lower pole like those of the first and second cleavage. Dean says, however, that "in depth and lateral extension its furrows are entirely similar to those of earlier cleavages."

Fourth cleavage.—The fourth cleavage is, as stated by Dean, "again a vertical one." The eight grooves forming this cleavage usually occupy the position shown in Fig. 20 (Plate XVII). In all the eggs examined but few were found which did not in general conform to this pattern. These few showed variations which resemble Fig. 5 or Fig. 19. The furrows of this cleavage extend to the equator of the egg and here fade out. In the egg shown in Fig. 5 the times and points of origin of some of these grooves are recorded.

Although, as stated, variations are sometimes found, it is of interest to note that these are far less numerous than those observed in Amia at a corresponding stage. Again, the fourth cleavage of Amia is usually circular, while that of Lepidosteus is most frequently vertical, the latter showing a tendency toward the Teleostean pattern. This cleavage in Acipenser, Amia, and Lepidosteus marks an interesting phase of the development, since it is the first to result in the formation of small cells at the active pole of the egg. A vertical section of this stage is shown in Fig. 35 (Plate XVIII), its plane being shown by the dotted line in diagram 24. The direction of the ensuing cleavages is indicated by the elongated nuclei.

Fifth cleavage.—With the formation of the fifth cleavage interesting changes are introduced. It comprises two distinct sets of grooves. The first set appears on the surface, as shown in Fig. 15 (Plate XVII), recalling the conditions observed in Amia. Often the grooves pass as shown in Fig. 15, in which case they give rise to a circular cleavage. More frequently it is of a mixed character, some grooves passing in vertical planes. The second set is confined to the four central cells and is not apparent on the surface; these four cells are divided in horizontal planes, as indicated in Figs. 35, 36 (Plate XVIII). The reconstruction of an egg in this stage gives Fig. 29, in which the elongated nuclei indicate the planes of the grooves constituting this cleavage. The variations from this pattern are numerous, and no attempt has been made to record them. If one were to offer a type, it would be that given in Fig. 29.

If this cleavage of Lepidosteus be compared with that of Amia, on the one hand, and the Teleost, on the other, some interesting facts are revealed. In order to make the comparison lucid we must recall that the fourth cleavage of Amia is usually circular. Whitman and Eycleshymer, speaking of this cleavage, state that "it represents, strictly speaking, eight distinct grooves, one for each of the primary segments, but these usually run together in such a way as to form a continuous groove, encircling the pole and bounding a polar field which may have a circular or oval form. This circular groove looks externally as if it might actually cut off polar segments, but, as an examination of sections will show, the groove descends more or less obliquely, or centripetally, so that when completed its inner deeper portion would be lost in confluent vacuolar spaces." The fifth cleavage, then, in Amia cuts off eight cells; that of Lepidosteus, four; that of the typical Teleost, four. This fact is emphasized because it is another point in the ontogeny of Lepidosteus which shows a closer resemblance to the Teleost than does Amia.

Later cleavage.—The next cleavage, which might be designated as the sixth, divides the eight central and the twenty-four marginal cells. The divisions are in general vertical, but many variations appear. I am able to confirm the statement of Dean that "the only cell divisions which were noted as generally constant were those of the marginal cells." Dean's statement that "its furrows extend no further than the margin of the cell cap" is not confirmed. Indeed, these furrows extend well down into the equatorial region, as depicted in Figs. 8–10. Vertical sections of these stages are represented in Figs. 37, 38; the former is taken from the margin of the blastodisc, while the latter passes through its center. The blastodisc is now in general two layers of cells deep, although some of the cells are now entering upon the seventh cleavage, as indicated by the horizontal division of one of the cells in the lower row. The segmentation cavity is poorly defined, being represented merely by intercellular spaces. The periblastic nuclei are most numerous at the center and are here undergoing active division, contributing one derivative to the cell cap, quite unlike that of the Teleost, but similar to the process in Amia.

After the sixth cleavage no attempt was made to follow the succession of grooves as presented by the living egg. The detailed study of the later stages is exceedingly difficult, owing to the fact that as cleavage goes on its character, as a whole, is somewhat modified. In the earlier stages of growth there are distinct periods of rest and activity; in the later stages this rhythm is gradually lost and the egg passes from a stage of rhythmical growth to that of continuous growth. As to the extent of the marginal grooves in the later stages, it may be said that they always terminate in an equatorial zone as depicted in the various figures. In Figs. 9, 10, 16, 17, 18 (Plate XVII), two series of later stages are represented. The eggs examined in these stages were from three different lots of preserved material and comprised several hundreds, yet in all this material there was never found an egg which conformed to the descriptions or illustrations given by Balfour and Parker, Beard, or Dean.

In the later stages of cleavage one frequently observes that the cells at one side, or margin, of the blastodisc are much smaller than those on the opposite side. Such conditions were found in the eggs represented in Figs. 16, 17, 18. While there is no question as to the existence in many eggs of areas of accelerated cell-division, I have been unable to determine whether or not these areas bear a definite and fixed relation to the embryo.

The section represented in Fig. 39 (Plate XVIII) is taken in a meridional plane through the blastodisc of an egg in the stage shown in Fig. 8 (Plate XVII). It will be seen that another set of horizontals has considerably increased the thickness of the cell cap, which is as yet made up of loosely scattered cells. A number of the intercellular spaces in this particular egg have become confluent below the body of the cell cap, thereby giving rise to a fairly well-defined segmentation cavity. The periblast forming the floor of the cavity continues to bud off cells, not only from its margin, as in the Teleosts, but irregularly over its entire surface. In none of the eggs at this stage has the writer found the "entire floor to be smoothly and distinctly differentiated," as stated by Dean. In Fig. 40 a transverse section of this same stage is shown; the section passes in a horizontal plane, midway between the equator and the upper pole, and shows the depth of the cleavage grooves at this period. The vertical section shown in Fig. 41 (Plate XVIII) is taken from an egg in the stage shown in Figs. 9, 18 (Plate XVII). Few noteworthy changes have occurred other than the increase in thickness of the cell cap.

In Fig. 42 a section is shown from an egg in the stage represented by Fig. 10. The plane of section is intermediate between horizontal and vertical, and shows an apparent shortening of the conical portion of the periblast. When contrasted with the preceding section, one notes that the cell cap has become more disc-like, owing to its marginal expansion over the yolk. The cells constituting the body of the cell cap are small, spherical, and fairly uniform. Its outermost layer is now transformed into a layer of elongated cells which form the superficial epiblast. The lower layer is made up of yolk-laden cells which are scattered loosely in the irregular segmentation cavity. The floor of the segmentation cavity must be regarded as periblast plus its yolk-laden derivatives. Fig. 43 (Plate XVIII) is a diagrammatic representation of a transverse section in the equatorial region showing the depth and distribution of the cleavage grooves.

Throughout the stages thus far studied the peculiar conical prolongation of the periblast has undergone little or no change in either form or structure. It is therefore premature to speculate as to its significance. A study of the later stages will undoubtedly throw some light upon the function of this hitherto unknown structure.

SOME GENERAL REMARKS

Balfour and Parker first observed, described, and figured the cleavage of Lepidosteus. Their conception of its character is concisely stated in these words:

> We have observed several stages in the segmentation, which show that it is complete, but that it approaches the meroblastic type more nearly than in the case of any other known holoblastic ovum.
>
> Our earliest stage showed a vertical furrow at the upper or animal pole, extending through about one-fifth of the circumference, and in a slightly later stage we found a similar furrow at right angles to the first. We have not been fortunate enough to observe the next phases of the segmentation, but on the second day after impregnation the animal pole is completely divided into small segments, which form a disc, homologous to the blastoderm of meroblastic ova; while the vegetative pole, which subsequently forms a large yolk-sac, is divided by a few vertical furrows, four of which nearly meet at the pole opposite the blastoderm. The majority of the vertical furrows extend only a short way from the edge of the small spheres, and are partially intercepted by imperfect equatorial furrows.

Beard next described the cleavage phases stating that "the segmentation is very unequal, but in a sense complete. Eight furrows can be traced to the center of the lower pole. The attempt to segment the lower hemisphere is, however, soon given up, none of the eight furrows penetrate very deeply into the yolk, and none reach the center by a long way. They are only superficial furrows."

Dean more recently gives a description and delineation of the successive cleavages, and concludes that the egg is meroblastic. In the detailed accounts of the successive meridional and vertical cleavage grooves (pp. 16–19) he explicitly states and repeats that the grooves extend no farther than the margin of the blastodisc.

Beard still later affirms his former statement and adds these words:

> If Dean were correct, the segmentation in Lepidosteus would be to all intents and purposes that of Scyllium, whereas if the view Balfour and Parker and I took of it be the right one, it would form a link between that of a frog or newt on the one hand and that of a skate or dog-fish on the other.
>
> This being so, the question of fact becomes of some importance. Dean has certainly had good opportunities for making sure of the point and — my own have been equally good. So far as can be gathered from Dean's statements and from my own abundant material of this period of development, the crux of the matter lies in the mode of preservation. Dean appears to have made no use of osmic acid in his investigations. The only two reagents employed by me were Flemming's fluid and corrosive sublimate.
>
> Eggs preserved in the latter fluid show no signs of the eight furrows described by myself, unless any sublimate remaining in the superficial part of the egg be precipitated by some such reagent as baryta water, but all the eggs of the proper stage (Fig. 3 of Balfour and Parker's memoir) show either four or eight complete furrows reaching to the lower pole. It is so easy to see these furrows in eggs lying in alcohol, or in eggs passed through turpentine and then dried, that I have often demonstrated them to others.

The above brief review emphasizes the differences of opinion among the investigators, and leads one to ask if the cleavage of the egg of Lepidosteus shows such wide variations, now cleaving in a holoblastic fashion, and again following the meroblastic form. If these variations do not exist, wherein lies the explanation of these diverse statements? If, on the other hand, they do exist, how shall we interpret so remarkable and exceptional a cleavage, and what new light may it throw upon the problems of gastrulation and embryo formation?

It would seem advisable, before going farther, to point out certain possible sources of misinterpretation.

Although Balfour and Parker state that the segmentation is complete, I think all will agree that this statement should be qualified, since they likewise repeatedly assert that the furrows "nearly meet," and to the latter statement their illustrations conform.

In Beard's still later writings his former observations are reaffirmed. The statement, however, that "*all the eggs of the proper stage show either four or eight complete furrows reaching to the lower pole*," is not in accord with my findings. Further, so far as I am aware, no other vertebrate ovum exhibits such a marked regularity of cleavage.

The observations on the early stages by Dean are more detailed than those of previous writers, but unfortunately the descriptions and illustrations are made from material fixed in alcoholic picro-sulphuric. The figures given by Dean in his first plate recall the appearances which I have frequently observed in material subjected to the same treatment, but to consider these gross artifacts as normal appearances is a most serious mistake. Dean apparently later realized the unreliability of these surface views, since he states in his paper on Amia (p. 414) that he had an "opportunity to observe the living material, and to prepare the figures of those stages especially which in surface view (as my studies of Acipenser and Lepidosteus had taught) could not well be examined in the fixed material."

As stated in my preliminary note, I firmly believe that, had Dean made his drawings from the living egg and confirmed their correctness by the study of material fixed by a number of reagents, his results would have been in complete agreement with mine. This conviction is supported by the appearance of the marginal grooves shown in Dean's Figs. 8, 11 (Plate I), and further strengthened by a suggestion in a footnote (p. 18), which reads as follows: "The writer has found no segmentation stages in which the furrows extend much lower than the equatorial region of the egg. He does not, accordingly, confirm the note and figures of Balfour, and is inclined to believe that the total segmentation of Lepidosteus occurs only as a variation."

It would thus appear that the differences of opinion expressed by Balfour and Parker, Dean, and myself might possibly be explained, but to reconcile these with the views of Beard is impossible. The only conclusion is that either the observations, somewhere, embody most extraordinary errors, or that the cleavage of the egg of Lepidosteus is of heterogeneous character — a discovery which would be of no little theoretical import.

A comparative study of the cleavage among the Ganoids reveals in this small group of fishes a most interesting series of transitional forms. Beginning with the holoblastic egg of Acipenser which closely resembles the Amphibian egg, we pass to the modified holoblastic as exhibited by the egg of Amia, thence to the egg of Lepidosteus with its meroblastic tendencies, and from this the typical meroblastic condition of the Teleost is readily derived.

It is a curious and striking fact that in these three Ganoids the first three cleavages are vertical, there being an entire absence of the usual horizontal division occurring in the third cleavage of Amphibia. It is likewise of interest to note that in the egg of Acipenser, which approaches most nearly the holoblastic type, we find the greatest percentage of variations in the first, second, and third cleavages; some of these are so marked that one or more of the grooves which form the third cleavage may pass in a horizontal plane. In the modified holoblastic egg of Amia the variations are less frequent; rarely, if ever, does a groove of this cleavage pass in a horizontal plane. Variations in the positions of the vertical furrows are numerous; they may pass through the poles forming true meridionals or pass far to one side of the pole giving rise to a bilateral type (Whitman and Eycleshymer). In Lepidosteus, which approaches most closely the meroblastic condition, the variations in the third set are less frequent than in Amia. In nearly all cases the grooves pass parallel to those of the first or second, being rarely meridional. In the fourth cleavage there are also interesting transitions, passing from the horizontal of Acipenser ruthenus (Salensky) through the modified horizontal or circular of Amia (Whitman and Eycleshymer) to the vertical of Lepidosteus.

The detailed study of cleavage in certain invertebrate groups, notably the Annelids and Molluscs, has led some writers to regard similarities in cell-lineage as prophetic of relationship. E. B. Wilson well expresses this conception as follows: "When the comparative study of cell-lineage has been carried further, the study of cleavage stages may prove as valuable a means for the investigation of homologies and of animal relationships as that of the embryonic and larval stages." Impressed by the striking homologies in the cleavage of the above forms, I was led at first to search among the vertebrates for similar means of determining relationships. Thus far, however, the facts do not justify the assumption that similarities in the cleavage of vertebrate ova are indicative of relationship.

It is therefore obvious that I cannot agree with Dean that "similarity in the mode of the first four cleavages" is evidence of kinship between Lepidosteus and the Teleost. Indeed, the danger of basing speculations regarding relationships upon similarities of cleavage are only made more apparent by my studies. As another illustration I find that Dean assumes from the study of the structure of recent and fossil Amioids that Amia is the "most Teleostean Ganoid." A study of its early development led to the belief that the egg is meroblastic, and this, regarded as an established fact, is taken by the author as confirming its close relationship to the Teleost. As a matter of fact, recent researches show the egg to be holoblastic; consequently the claim that it is the "most Teleostean Ganoid" receives no support from early ontogeny.

A comparison of the rate of development in Lepidosteus as recorded by the several investigators reveals most striking differences. A tabulation of the times at which development reaches well-marked stages is given below:

Stage of Development	Agassiz	Balfour and Parker	Beard	Fülleborn	Dean	Eycleshymer
Late cleavage, cell cap, 100-200 cells	....	48 hrs.+	5-6 hours	....	25 hours	5-6 hours
Gastrulation complete, blastopore closes	....	....	24 hours	....	46 hours	28 hours
Optic vesicles distinguishable	....	60 hours	....	....	80 hours	45 hours
Embryo hatches	8 days	8 days	7 & 9 days	3 days+	8-9 days	6 days
Pectoral fins appear	10 days	10 days	....	....	....	7½ days
Pelvic fins appear	19 days	14 days	....	....	....	13 days
Yolk-sac absorbed	26 days	26 days	....	....	41+	22 days

Balfour and Parker found that on the third day (tabulated as 48 hours+) after impregnation the animal pole is completely divided into small segments, which form a disc similar to the blastoderm of meroblastic ova. Beard states: "The segmentation is very rapid and a cap of small cells is formed in the course of 5 or 6 hours." According to the data given by Dean (p. 66, Fig. 12, and Plate I, Fig. 12) this stage of development is not attained in less than twenty-five hours. I find that it is reached in five or six hours as recorded by Beard.

The closure of the blastopore, which marks the end of gastrulation, takes place, according to Beard's and my own observations, in about twenty-six to twenty-eight hours; while Dean finds that nearly twice this length of time is necessary for the egg to reach the same stage. Again, as pointed out in a preceding paragraph, a comparison of ages at the time of hatching shows variations, ranging from three days to nine days.

The above considerations lead us to infer that environmental (thermic?) conditions may so modify the rate of growth that a given degree of differentiation may be attained in a fraction of the usual time without inducing pathological changes.

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EXPLANATION OF PLATES

PLATE XVII

Figs. 1–8, 11–15, 19, 20 are drawn from living eggs; the remainder are drawn from eggs fixed and hardened in 10 per cent formaline. The numerals affixed to the cleavage grooves indicate the time at which the grooves reached the points indicated by the dotted lines.

Fig. 1.— Profile view of the unsegmented egg immediately after fertilization, showing natural colors of egg. × 10.

Fig. 2.— Profile view of an egg one hour and forty-eight minutes after fertilization, showing the position and extent of the first vertical groove. × 15.

Fig. 3.— Profile view of the same egg two hours and thirty minutes after fertilization. The first vertical has extended slightly beyond the margin of the calotte, while the second has barely reached the margin. × 15.

Fig. 4.— Profile view of the same egg three hours after fertilization, showing the position and extent of the first three sets of verticals. × 15.

Fig. 5.— Profile view of the same egg three hours and thirty-five minutes after fertilization, showing the times at which the grooves of the fourth cleavage appeared and their position with reference to the other grooves. × 15.

Fig. 6.— Profile view of the same egg four hours and ten minutes after fertilization, showing the division of the eight central cells, also the addition of more meridionals. × 15.

Fig. 7.— Profile view of another egg about five hours after fertilization. × 15.

Fig. 8.— Profile view of egg five hours and thirty minutes after fertilization. × 15.

Fig. 9.— Profile view of egg about six hours after fertilization. × 15.

Fig. 10.— Profile view of egg in late blastula, about seven hours after fertilization, showing extent of cleavage just before gastrulation. × 15.

Figs. 11–15 represent a series of successive cleavages as viewed from the upper pole. × 15.

Figs. 16–18 represent succeeding stages as observed in the preserved material.

Figs. 19, 20 show some of the more usual conditions observed in the formation of the fourth-cleavage grooves. × 15.

PLATE XVIII

Figs. 21–24 are diagrams of cleavage phases showing the lines of section of these respective stages.

Fig. 25 is a horizontal section at the level of the nuclei of the four-cell stage, showing the planes of the elongated nuclei preparatory to the third cleavage. × *ca.* 8.

Figs. 26–28 represent a series of horizontal sections of the blastodisc of an egg in which the grooves are well marked at the level of the nuclei, but have not yet appeared on the surface. An intercellular space is represented which is the beginning of the segmentation cavity. × *ca.* 9.

Fig. 29 is a diagram of an egg in the fourth cleavage, taken in a horizontal plane, at the level of the dividing nuclei, and showing the plane in which the nuclei are elongating for the ensuing cleavage. × *ca.* 10.

Fig. 30 is a vertical section of a mature ovarian egg, showing the surrounding membranes, the micropylar orifice, the germinal vesicle, and the peculiar relation of the finely granular germinal portion to the coarsely granular nutritive portion. The yolk has not been completely filled in. × 18.

Fig. 31 represents a horizontal section of a similar stage, somewhat below the level of the equator, and, like the preceding, shows the relation of the finely and coarsely granular portions. × 18.

FIG. 32 represents a section of an egg in first cleavage, taken along the dotted line which passes through the center of the egg in Fig. 21. × 18.

FIG. 33 is a section of the same egg, but taken nearer the margin of the blastodisc, as indicated by the second dotted line of Fig. 21. × 18.

FIG. 34 represents a vertical section of an egg in third cleavage, the section being taken along the dotted line in Fig. 23. × 18.

FIG. 35 represents a section of an egg in fourth cleavage along the dotted line in Fig. 29. The section, instead of being vertical, passes obliquely. It will be noticed that the plane of nuclear elongation is that shown in Fig. 29. × 18.

FIG. 36 is a section of an egg in the same stage, but passes at right angles to the preceding. The horizontal division of the central cells is shown. × 18.

FIGS. 37, 38 represent sections of an egg in the sixth cleavage. The former is taken through the margin of the blastodisc, while the latter passes through the center. × 18.

FIG. 39 represents a vertical section of an egg in about the seventh cleavage. The thickness of cell cap, the segmentation cavity, the budding off of cells from the periblast, etc., are indicated. × 18.

FIG. 40 represents a transverse section of an egg in the same stage as the preceding. The section is taken midway between the upper pole and the equator, and shows the depth of the cleavage grooves. × 18.

FIG. 41 represents a vertical section of an egg in the stage shown in Figs. 9 and 18. Few noteworthy changes have occurred beyond the increased thickness of the cell cap. × 18.

FIG. 42 represents a section of an egg in the stage shown in Fig. 10. The apparent discontinuity of the conical portion of the periblast is due to the obliquity of the section. The stage is that of late blastula. The body of the cell cap is made up of small spherical cells. The outermost layer is differentiated into the superficial epiblast, while the lower layer consists of yolk-laden cells loosely scattered throughout the segmentation cavity. × 18.

FIG. 43 is a diagram of an egg in the same stage as 42, but taken in a horizontal plane at the level of the equator, showing the depth of the cleavage grooves at this time. × 18.

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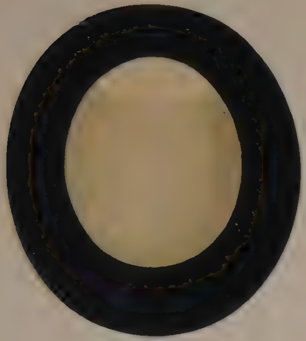

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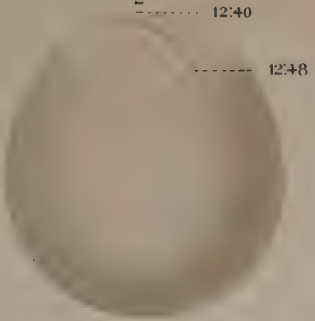

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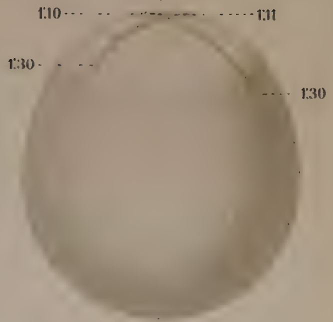

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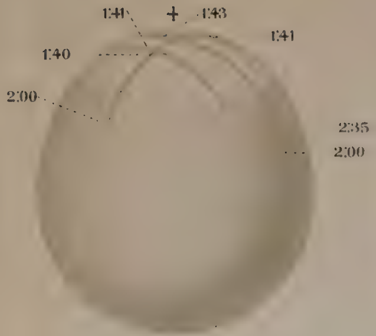

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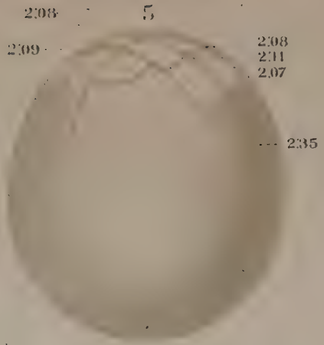

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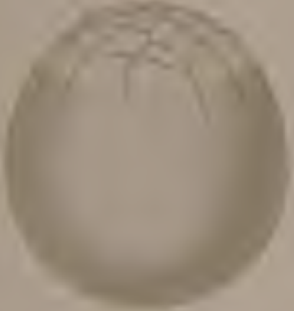

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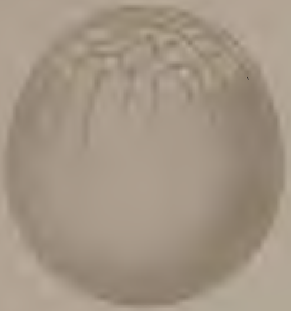

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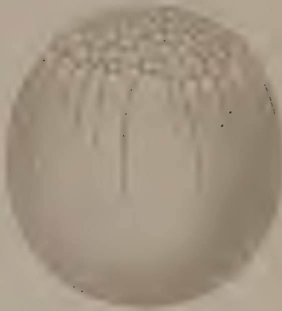

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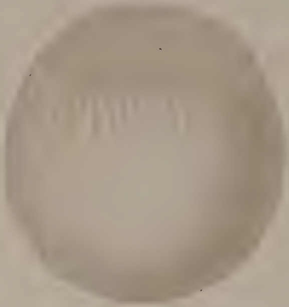

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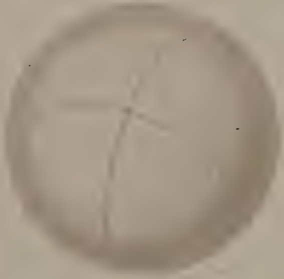

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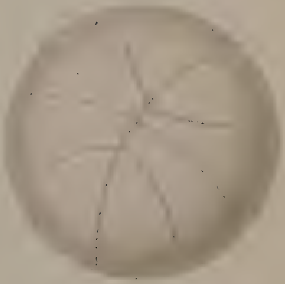

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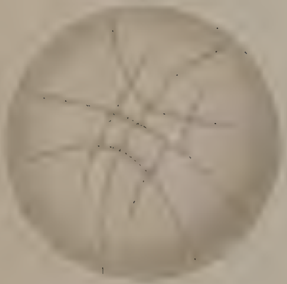

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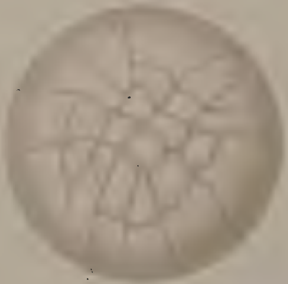

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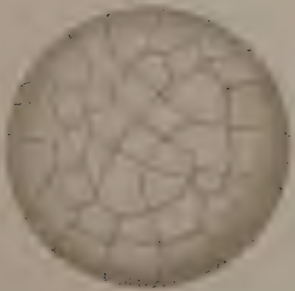

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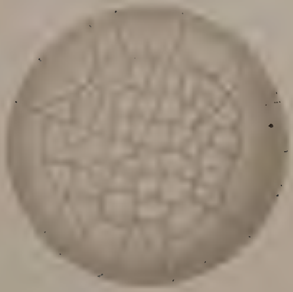

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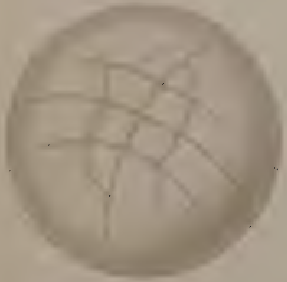

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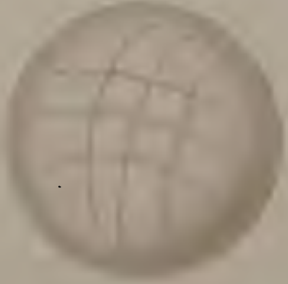

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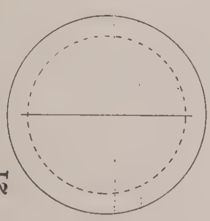
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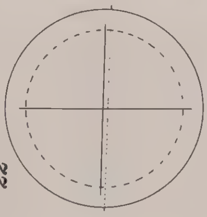
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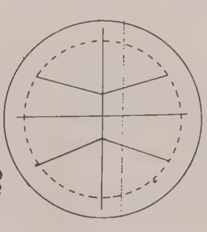
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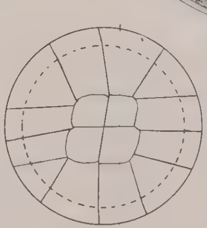
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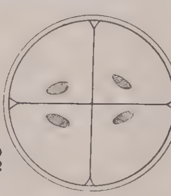
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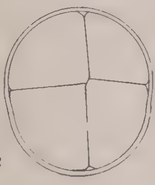
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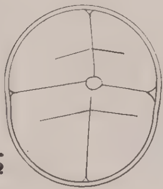
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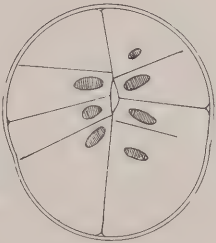
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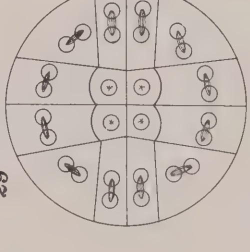
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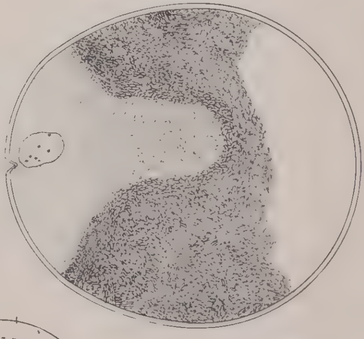
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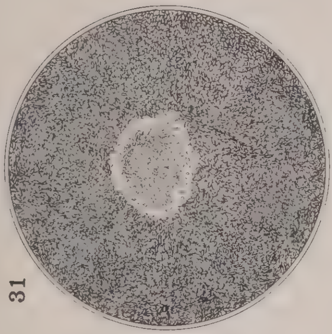
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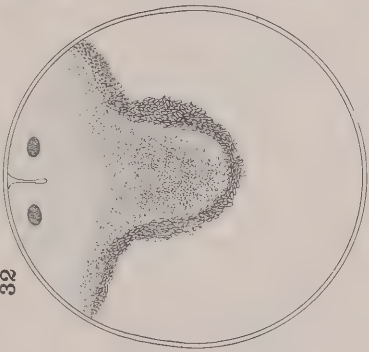
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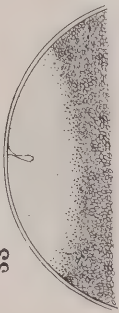
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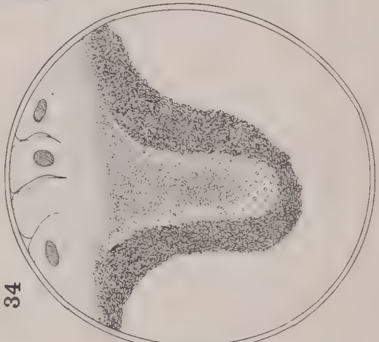
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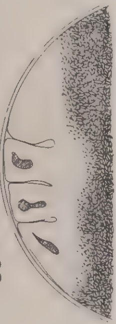
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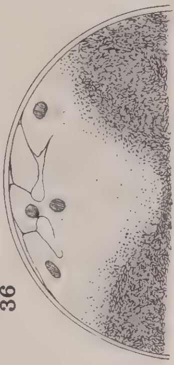
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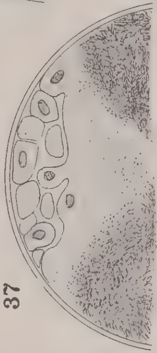
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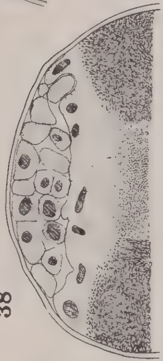
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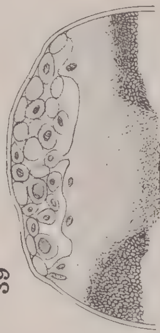
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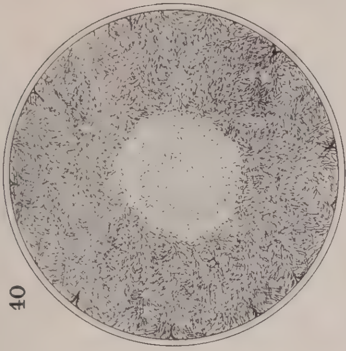
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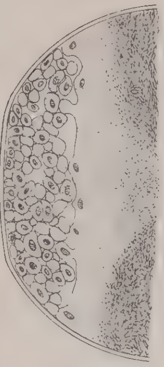
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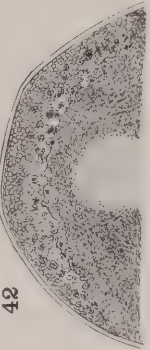
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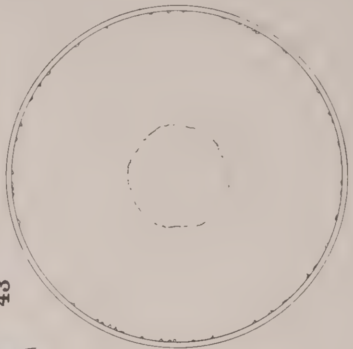
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